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INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DISSABS, DDFB, DDFU, DGENE, DRUGB, DRUGMONOG2, ...' ENTERED AT 14:07:02 ON 23 JUN 2004

SEA GLUCOAMYLASE

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L1 QUE GLUCOAMYLASE  
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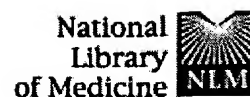
FILE 'CAPLUS, BIOSIS, SCISEARCH, BIOTECHDS, FSTA, PASCAL, EMBASE,  
MEDLINE, LIFESCI, WPIDS, JICST-EPLUS, BIOTECHNO, AGRICOLA' ENTERED AT  
14:08:08 ON 23 JUN 2004

L2 1 S L1 AND (POSITION 402 OR AMINO ACID 402 OR TYR402 OR Y402)

L6 ANSWER 143 OF 143 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
 ACCESSION NUMBER: 1991-01384 BIOTECHDS  
 TITLE: Expression and routeing of human lysosomal alpha-glucosidase  
 in transiently transfected mammalian cells;  
**glucoamylase** gene cloning and expression in  
 COS-1 and HeLa cell culture; potential application in  
 glycogenosis type-II gene therapy  
 AUTHOR: Hoefsloot L H; Willemsen R; Kroos M A; Hoogeveen-Westerveld  
 M; Hermans M M P; \*Reuser A J J  
 LOCATION: Department of Cell Biology and Genetics, Erasmus University,  
 P.O. Box 1738, 3000 DR, Rotterdam, The Netherlands.  
 SOURCE: Biochem.J.; (1990) 272, 2, 485-92  
 CODEN: BIJOAK  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB cDNA encoding human acid alpha-glucosidase (**glucoamylase**,  
 EC-3.2.1.3) was cloned in vector plasmid pSG5 and used to transform COS-1  
 and HeLa cells. mRNA was synthesized from the phage T7 promoter in front  
 of the cDNA insert, and used to direct protein synthesis in a  
 reticulocyte translation system. Only translation of sense mRNA led to  
 protein production. Plasmid pSHAG1 did not encode a functional enzyme,  
 due to an Arg residue replacing a Trp residue at **position**  
**402**. The mutation did not affect enzyme production, but  
 interfered with post-translational modification and intracellular  
 transport of the precursor. Pulse-chase experiments suggested that the  
 precursor was denatured. A Trp-402-containing enzyme (encoded by plasmid  
 pSHAG2) was processed properly, was active, and reached the membrane and  
 the medium. The proteins formed in the absence and in the presence of  
 microsomes corresponded in their mol.weight to previously identified  
 unglycosylated and glycosylated precursors of **glucoamylase**,  
 obtained by translation in vitro of total RNA from human fibroblasts.  
 The enzyme may be useful in gene therapy of e.g. glycogenosis type II.  
 (36 ref)

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L2 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
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## Expression and routing of human lysosomal alpha-glucosidase in transiently transfected mammalian cells.

Hoefsloot LH, Willemsen R, Kroos MA, Hoogeveen-Westerveld M, Hermans MM, Van der Ploeg AT, Oostra BA, Reuser AJ.

MGC-Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands.

Previously isolated lysosomal alpha-glucosidase cDNA clones were ligated to full-length constructs for expression in vitro and in mammalian cells. One of these constructs (pSHAG1) did not code for functional enzyme, due to an arginine residue instead of a tryptophan residue at amino acid position 402. The mutation does not affect the rate of enzyme synthesis, but interferes with post-translational modification and intracellular transport of the acid alpha-glucosidase precursor. Using immunocytochemistry it is demonstrated that the mutant precursor traverses the endoplasmic reticulum and the Golgi complex, but does not reach the lysosomes. Pulse-chase experiments suggest premature degradation. The Trp-402-containing enzyme (encoded by construct pSHAG2) is processed properly, and has catalytic activity. A fraction of the enzyme is localized at the plasma membrane. It is hypothesized that membrane association of the acid alpha-glucosidase precursor, as demonstrated by Triton X-114 phase separation, is responsible for transport to this location. Transiently expressed acid alpha-glucosidase also enters the secretory pathway, since a catalytically active precursor is found in the culture medium. This precursor has the appropriate characteristics for use in enzyme replacement therapy. Efficient uptake via the mannose 6-phosphate receptor results in degradation of lysosomal glycogen in cultured fibroblasts and muscle cells from patients with glycogenosis type II.

PMID: 2268275 [PubMed - indexed for MEDLINE]

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